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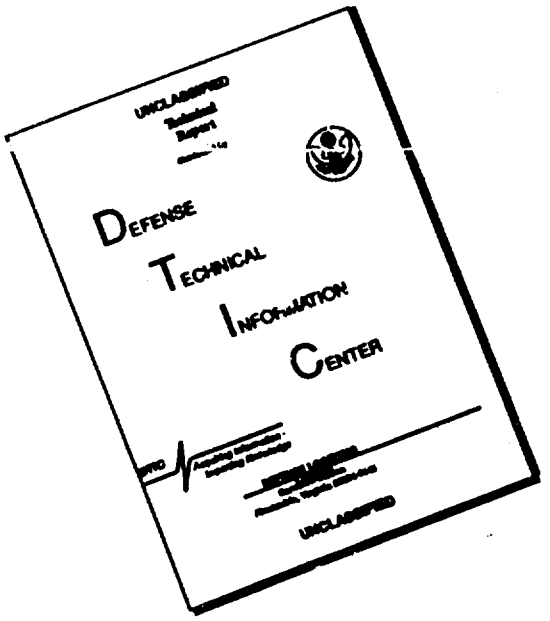

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SIMULTANEOUS [illegible] OF PLASMA VOLUME AND TOTAL ERYTHROCYTE QUANTITY WITH [illegible]-ALBUMIN, AND A CONTRIBUTION ABOUT THE RELATIONSHIP BETWEEN [illegible] AND BODY HAEMATOCRIT DURING HAEMATOLOGIC DISEASES

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Simultaneous Determination of Plasma Volume and Total Erythrocyte Quantity with Cr^{51} and I^{125}-Albumin, and a Contribution About the Relationship between Peripheral and Body Hematocrit During Hematologic Diseases

(The following is the translation of an article by H. Huber, K. Keller and G. Miccabona, Isotope Laboratory of the Medical Clinic of the University of Innsbruck, that appeared in the German language periodical Blut (Blood), Vol. 13, no. 1, 1966, pages 1-9. Translation performed by Constance L. Lust.)

Blood volume measurements are routine in many isotope labs. Usually the total erythrocytes or plasma volume are determined, and by using "corrected" hematocrit values the blood volume is determined (1, 2). A series of illnesses was reported during which the use of the "correcting-factor" lead to wrong results, so that a simultaneous measurement of the two blood volume parts would be advantageous (1, 3-6).

Total erythrocyte mass is determined mostly, by using Cr^{51}-labelling of erythrocytes; Albumin-I^{125} is used for plasma volume. Because of the similar energy spectra simultaneous determinations of these two isotopes may lead to difficulties. It is possible to do this if the short-lived I^{132} is used instead of I^{125} (I^{132} $T_{\frac{1}{2}}$ = 2.3 hrs). For this reason this is expensive and not generally useful for routine work. Plasma volume was measured with $Cr^{51}Cl_3$; total erythrocytes $Na_2Cr^{51}O_4$ (4); this requires a thorough separation of erythrocytes and plasma in order to measure radioactivity. Determining life of the erythrocyte is fraught with errors as is a plasma volume determination with albumin^{131}. If erythrocytes are labelled with P^{32}, than one can do I^{131} albumin for PV, but beta rays are more difficult to measure. This isotope is also eluted rapidly (1). One can use Fe^{59} labelling and get PV by extrapolation; Cr^{51} may be used simultaneously. Plasma clearance of Fe^{59} is rapid, exponential (7), so that graphic extrapolation may lead to difficulties. Labelling erythrocytes with dyes is used frequently to get PV, but this method has disadvantages (1).

We encountered trouble in the technique of the simultaneous determination. The present method, however, gave us good results, it is simple and needs a minimum of equipment. It is used to emphasize the relation between peripheral and body hematocrit. A group of patients with anemias were used along with controls. One patient had an enlarged spleen and was spleenectomized, one had polythemia.

Methods

Circulating Erythrocytes

About 20 ml. venous blood was collected and mixed with 0.5 ml. heparin (2500E), and centrifuged at 2000 rpm for 5 minutes. The supernatant plasma was discarded and 80-100 microcuries of Cr^{51} (as $Na_2Cr\ O_4$)

was added (Radiochemical Centre Amersham spec. act. 1.32-1.51 mc/micro gram). Incubation was [illegible] minutes. Excess Cr^{51} was removed by washing 3x with normal saline. The labelled erythrocytes were suspended in 25 ml. physiological [illegible] exactly 20 ml. of this was injected intravenously into a subject. [illegible] ml. of the labelled erythrocytes were used as standard, and were [illegible] 100 ml. after lysis with saponin. Ten minutes after injection [illegible] samples were drawn, usually 4, and collected in a heparinized [illegible] The hematocrit was done with the microhematrocrit method. [illegible] added to a part of the sample, and 2 ml. of this hemolysate [illegible]. The rest was centrifuged, and the plasma served in the PV determination. An automatic sample changer, liquid scintillator was used to count the radioactivity; 5000 impulses (counts) were counted, so that the counting error was - 3%. The usual formulae were used to calculate total erythrocytes (2, 8). No correction was required in I^{125} counting method.

Plasma volume:

For PV determination chromatin I^{125} was used (Farbwerke Hoechst AG spec. act. 0.01[illegible] mc/g iodinated albumin). The stock solution was diluted with saline so that a 10 ml. volume had 10 micro curies and this was injected. The injection followed the marked red-cells, the volume syringe was weighed before and after. Blood samples were taken (heparinized) in 10-15 minute intervals for one hour. The samples for determination were taken from whole blood; erythrocyte volume and hematocrit in quatruplicate. The remaining blood was centrifuged at 3000 rpm for 10 minutes and used for I^{125} measurement (2 ml.). The I^{125} albumin standard was 1 ml. of that injected; this was diluted to 100 ml. with a 0.1% protein solution. The activity measured in a counter was plotted as a semi-log plot against time. Time 0 was determined by fitting the line to the best points on the graph. PV was calculated with the usual formulae (2, 8). Since we did not use hemolysate [illegible] no correction for Cr^{51} counts was necessary.

Hematocrit Determination:

In every case venous and peripheral hematocrit were done and the results corresponded with those of Dacie and Lewis (2). The microhematocrit method was performed; the blood was centrifuged at 12,000 g for 5 minutes. We did apply the correction factor for plasma error, since this was very small in this case (9).

Calculation of body hematocrit and "correction-factor". Thefollowing formulae were used:

$$EM = \frac{\text{[illegible]}}{\text{[illegible]} + PV} \times 100; \quad f = \frac{KHK}{pHK}$$

EM = total erythrocyte mass
PV = plasma volume
f = correction factor
KHK = body hematocrit (in %)
pHK = peripheral [illegible]crit (in %)

Persons used in the investigation:

A total of 35 persons were used in groups as follows:

a) Control group: 10 people who had no hematological illnesses, or any other illness leading to blood volume alteration (10). Five had bronchus carcinomas; three had degenerative cardial illness without any signs of decompensation; two had chronic tonsilitis.

b) Patients with anemias without spleenomegaly. Ten patients with variously caused anemias were used. They had no enlarged spleens. Iron-deficiency anemias without acute, heavy blood losses (5 cases), patients with acute myelogenous leukemia (3 cases) and 1 patient had lymphosarcoma with aplastic anemia and congenital spherocytosis.

c) Patients with spleenomegaly as well as some who had spleens removed: 10 patients with spleenomegaly, as well as some with chronic lymphoadenosis (4 cases), 2 with osteomyaloscherosis, 1 with cirrhosis of the liver and tumor of the spleen.

Three patients were studied after removal of spleens, necessitated by ostermyaloscherosis (weight of spleen 1520-3200g); 3 had idopathic thromopenic purpura (wt. spleen 220 g). One had an operation 6 weeks previously, the others 1.5 years and 16 years respectively.

d) Patients with polycyma: Two had polyzyma were without enlarged spleen (peripheral hematocrit 65 + 69% respectively); one also had osteomyaloscherosis, and been spleenoctomized 2 years earlier (hematocrit 40%). This patient was also used in group c.

Results

1) Energy Spectra of Cr^{51} and I^{125} and the simultaneous measurement of these isotopes.

The differences in the energy spectrum of I^{125} and Cr^{51} are illustrated in Figure 1. In the I^{125} channel there was a part of the low-energy secondary Cr^{51} rays. No correction factors were necessary, since I^{125}-albumin in plasma was determined after the erythrocytes had been removed. Cr^{51} was measured in whole blood, whereby the I^{125} activity in the Cr^{51} channel was less than 0.1%. The effeciency of counting was 2% of the input under these experimental conditions; I^{125} was 30% of input.

2) The relationship between peripheral and body hematocrit is demonstrated in Figure 2. A close dependence was observed between the two parameters in the control subjects, patients with anemia without enlarged spleens and those with polycema ($r = 0.991$). For those ill with spleenogalies of various origin in 8 of 10 cases the body hematocrit was significantly elevated, if the results are compared to the rest of the groups (figure 2).

3) Correction factors for determining body hematocrit from the peripheral hematocrit values. The correction factors became apparent form Figure 3. In the control group, patients with anemias without enlarged

spleens, etc., comparable results were found. For two patients with the severest anemia in respect to acute leukosis (peripheral hematocrit 19 and 13.5%) f dropped to 0.740 and 0.745 respectively. This result was not included in our statistical calculations, but must be if larger groups of patients are involved. (The patients were under treatment of daily doses of corticosteroid of 200 mg methylprednisolone at the time of this investigation).

The 10 patients that had spleenomegalies had f values significantly higher ($p < 0.001$). The highest value was observed with the patient with the biggest spleen tumor, (25 cm below ribs) (f = 1.120). Four patients with spleen tumors (15 cm below ribs) had f value about 1; the remaining 6 patients with slightly enlarged spleens the f value was mostly below 1.

Three patients were studied after spleens were removed. One 6 weeks previously; he had osteomyeloscherosis and the f value was 0.824 and was below the control group (fig. 3). For the other two patients -one with idopathic thromopenic purpura, one with polyczema vera- were operated on 1.5 years and 16 years previously respectively. f was in the normal range (0.88 and 0.90).

Discussion

The isotopic method described allows one to do a simultaneous determination of erythrocyte mass and PV in a convenient way. Prerequisits for using this method are adequately large differences in the energy spectra of the isotopes to be used. Cr^{51} which is used to label erythrocytes decomposes by electron capture; the low energy gamma rays of Cr^{51} lie in the region of 325 Kev. The photon maximum for I^{125} is at 35 Kev (Fig. 1). This large difference in the energy of the emitted gamma rays allow for good separation of the two isotopes. Measurement of these simultaneously requires only simple equipment, needing only a discrimminator. It is easy to measure the "life-time" of erythrocytes, and the surface-areas at the place of hemolysis is done without difficulty, because the I^{125} activity is minimal in the Cr^{51} channel (below 0.1%). In contrast to the often used I^{131}-albumin this iodine isotope also has the advantage of better counting efficiency and less isotope effect (11). The dose of I^{125} is usually less and therefore the labelled protein is in less danger of being denatured (12). Further it has a relatively long half life of 60 days.

By determining erythrocyte mass and PV simultaneously it is possible to determine blood volume directly, also the body hematocrit may be determined which may be of interest in reference to peripheral hematocrit (1, 3-6, 13). The vessel volume, wherein the plasma circulates, is usually larger than that of the red cells (1, 14). The relationship of red cells to plasma in the circulating total volume (body hematocrit) compared to the peripheral hematocrit is usually lower than 1 (1, 4, 6, 13 etc.) This report shows the constancy of this relationship (correction factor) in normal persons and substantiates the results of a series of authors who all used different methods for determining red-cell mass and PV. The correction factor, f, in normals was 0.899 with a standard deviation of 0.023 (fig. 3) and is

comparable to the values of (13) 0.910 ± 0.026; (4) 0.896 ± 0.039 (6) 0.902 ± 0.022. "f" was similar to normals for patients with anemias, etc, provided no enlarged spleen was present. This also agrees with other authors (1, 13). A series of hypotheses were proposed about this constant relationship, even when marked hematocrit alterations were reported (1, 6, 13).

Since red blood cell concentration is greater in spleen blood than in the other vessels (3, 4, 12, 15), a large increase in the size of spleen could lead to increased values of f. The results in the literature are in opposition to this concept. Rothschild et al (3) Fudenberg et al (4) found and increase, Chaplin et al (13) found f (correction factor) unchanged. The ill subjects with spleenomegaly a higher body hematocrit was needed in order to reach a certain peripheral hematocrit (fig. 2). f was 1.003 ± 0.051 in these subjects compared to 0.870 ± 0.017 for the patients with anemias without enlarged spleens (fig. 3). The f valve was higher in the former group as compared to those with smaller enlarged spleens. This agrees with Fudenberg et al (4). In studies on osteoscherosis (16) as well as spleenomegalies (17) it was observed that to reach a definite peripheral hematocrit in these patients, a doubling of the red cell mass was needed than in patients with enlarged spleens. This may be an important finding and indicates a build up of erthrocytes during spleenomegalies. As was calculated by Chaplin et al (13), large changes in distribution of red-cells are required to significantly elevate f values.

Only three patients were studied after spleenectomy, so that these results need substantiation in larger numbers of patients (fig. 3). One had the operation 6 weeks previously and f for this patient was below the region of the control group. This result agrees with Fudenberg et al (4) where the decrease was explained by enlargement of the portal vessells with increased capillary blood volume. This held more plasma than the larger vessells, as was shown in a series of studies(1, 14, 21). Even in dogs spleenectomy caused a lowering of the correction factor (22). For the patients whose spleenectomy was 1.5 and 16 years ago f was in the control range. This also was observed by Fudenberg et al (4).

Blood volume determinations are of clinical interest in a variety of situations and can be of value in hematological diseases (10). With spleenomegalies of different origin (4, 16, 19), anemias in the realm of chronic liver disease (23), acute and chronic kidney insufficiency with oliguria (24) and during several other diseases (6, 10) blood volume elevations were at least partially responsible for a decrease in hematocrits.

If BV measurements are done on normals or on patients with anemia without using the correction factor the results are usually high. The discrepancy of the measurement can be about 20%. Even though in most cases a useful measure of BV can be obtained from PV or erythrocyte mass and a peripheral hematocrit (must be corrected), for exact determinations the BV must be done directly simultaneously (1). This report shows that during spleenomegalies and even maybe after spleenectomy the correction factor (f) for hematocrit should be used. Similarly during liquid retention

during the course of corticosteriod therapy as well as with heart diseases 5) its use is indicated. This method allows one to do a simultaneous determination of erythrocytes and PV in a simple manner.

Summary

1. A simple method for the simultaneous estimation of plasma volume and total number of erythrocytes using Cr^{51} and I^{125}-albumin is described and its advantages over other methods of simultaneous estimation are discussed.

2. The relation between body hematocrit-peripheral hematocrit values is investigated in 35 people. In the control group made up of anaemia of different origin and severity but without enlargement of the spleen and polycythaemics the results were comparable with those using various other methods of blood volume estimations.

3. A highly significant deviation ($p < 0.001$) from the norm was shown in 10 patients with splenomegaly of various origin. Different results were obtained in 3 splenectomized patients.

4. The importance of the changes with regard to the anaemia of patients with splenomegaly and the value of blood volume estimations in various diseases of the blood is discussed in conclusion.

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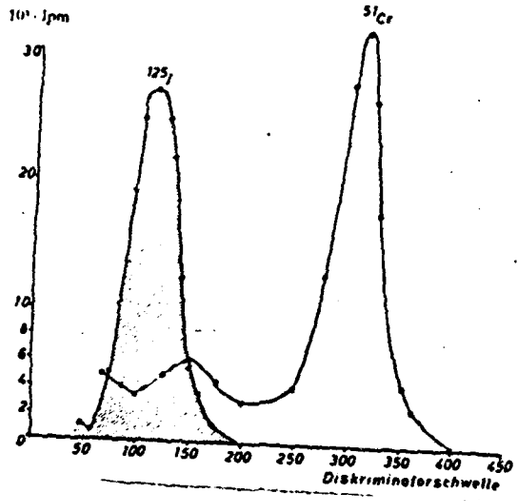

Figure 1
Energy Spectra of I^{125} and Cr^{51} high voltage 910 Cr^{51}, 1020 Cr^{51}

channel width 10v;
activity 1 micro curie Cr^{51}
0.05 uc I^{125}

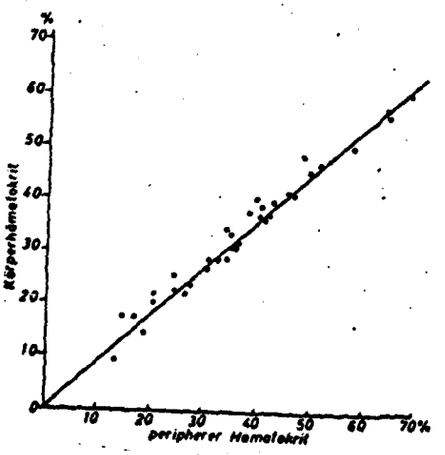

Figure 2

Relationship between body hematocrit and peripheral hematocrit (n = 35)
open circles - patients with splenomegaly
solid circles - patients with enlarged spleen

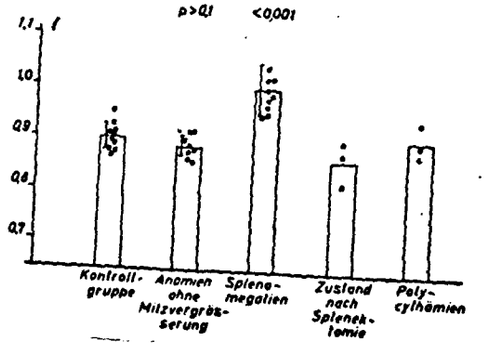

Figure 3 Correction factor "f" in the control group and various groups of patients